Standard Operating Procedure for Derivatization with Diazomethane

Scope

This process is to derivatized chlorinated herbicides to their methyl esters. This process is needed so that these analytes can be analyzed by GC/ECD or GC/MS.

Summary

Diazomethane is generated from the action of alkali on N –methyl- N–nitrous-p-toluene sulfonamide. Nitrogen gas passed through the diazomethane generator which transfers the solution of diazomethane to methyl-t-butyl ether in a collecting vial. The diazomethane is utilized for the esterification of the extracted chlorophenoxy herbicides.

Safety:

Diazomethane is a toxic carcinogen which can explode under certain conditions. The following precautions must be followed:

1) Use only a well ventilated hood - do not breathe vapors.

2) Use a safety screen.

3) Use mechanical pipetting aides

4) Do not heat above 90°C - **EXPLOSION** may result.

5) Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers - **EXPLOSION** may result.

6) Store away from alkali metals - **EXPLOSION** may result.

7) Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

8) The diazomethane generation apparatus used in esterification procedure produces micromolar amounts of diazomethane to minimize safety hazards.

Glassware:

A. 2 graduated centrifuge tubes that have openings for stoppers.

B. 1 screw topped collection vial in a cooling apparatus to maintain a 4ºC during the collection of diazomethane.

C. 2-holed rubber stoppers that fit into the centrifuge tubes.

D. Tubing and connections to assemble the diazomethane generator, see figure 1.

Reagents

A. Carbitol (Di(ethylene glycol) ethyl ether), Aldrich E455-0, 100 g bottle (refrigerated).

B. Diazald (–methyl-–nitrous-p-toluene sulfonamide), Aldrich D2,800-0, 100 g bottle (refrigerated).

C. Potassium Hydroxide, KOH, ACS grade.

D. Ethyl Ether, Nanograde un-preserved, peroxide-free (stored under hood).

E. Methyl-t-butyl ether, MTBE Nanograde (stored under hood).

F. 37% KOH: Weigh 37 grams of KOH into a 100 ml volumetric flask and bring to volume with nanograde water (store at 4°C).

G. Diazald soln. Dissolve ~~10~~ 5 grams of diazald in ~~100~~ 50 ml of a 50/50 solution by volume of ethyl ether and carbitol. This solution is stable for three months when stored at 4°C in an amber bottle with a Teflon lined screw cap.

Procedure

A. Diazomethane Preparation: Refer to Fig. 1.

1. Add a sufficient amount of ethyl ether to tube #1 so that the longer dip tube is immersed. (Leave ether in tube.) Place the stopper with connecting tubing on tube #1.

2. Add ~~5~~ 10 ml of MTBE to collection vial. Place vial in ice tub.

3. Add ~~2~~ 4 ml of the diazald solution to tube #2 (Use the 2mL Eppendorf

micro-pipette)

4. Add 4 ml of 37% KOH to tube #2.

5. Place the stopper and connecting tubing on tube #2 and the system exit tube into the MTBE in the collection vial in the ice tub. Set the nitrogen flow to 5-10 mL/minute. Allow the nitrogen to purge while the reaction is occurring and continue for 30 minutes.

6. Cap the collection vial and store in freezer in well of ice tub.

B. Esterification:

1. Add 250 μl of methanol to the tube containing the sample. (Use 100-1000 Eppendorf).

2. Add 0.5 ml of the diazomethane solution. (Use the 100-1000 Eppendorf micro-pippette) Samples should turn yellow after addition of diazomethane solution and remain yellow for at least 2 min. Repeat methylation procedure if necessary.

3. Cap the tube mix well and allow the reaction to occur for at least 30 minutes.

4. Destroy any unreacted diazomethane by adding 0.1 to 0.2 grams silicic acid to the sample tubes. Mix well.

4. Adjust the final sample volume to 5.0 ml using MTBE. Allow to stand until the evolution of nitrogen gas has stopped (approximately 20 mins.)

5. Store remaining diazomethane solution in freezer or dispose of properly.

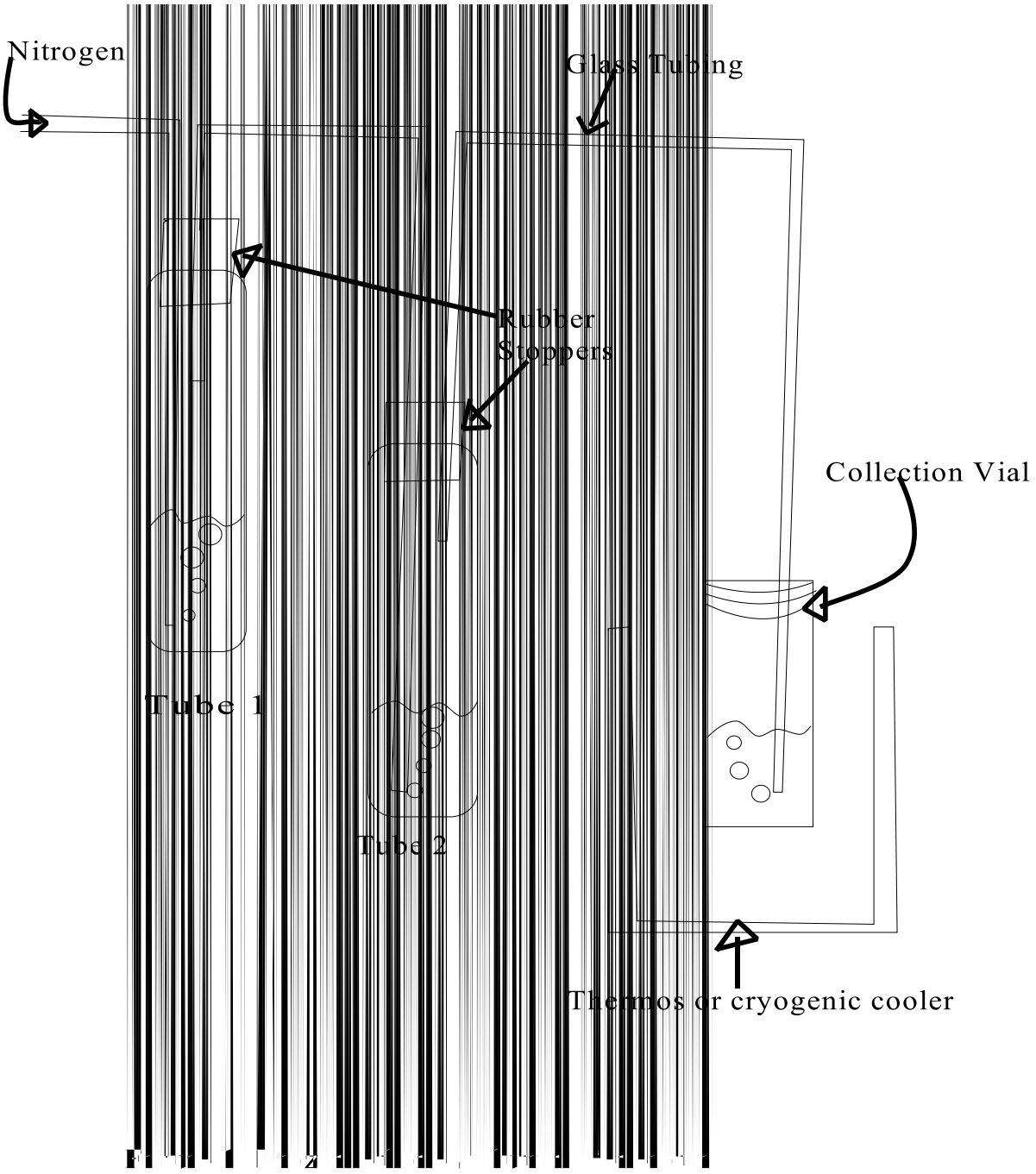
6. Wash tube #2.

7. Proceed with gas chromatographic analysis.

References

A. Aldrich, Tech. Bull, No. AL-180, Diazald, MNNG and Diazomethane Generators.

B. Fales, H.M. and T.M. Jaouni, 1973. Simple device for preparing ethereal diazomethane without resorting to co-distillation. Analyt. Chem. 45: 2302-2303.


Nitrogen
Glass Tubing
Rubber
Stoppers
Collection Vial
Tube 1
Tube 2
Thermos or cryogenic cooler